

Immunologic analysis and serum heavy metal levels in exacerbated Tourette syndrome

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Tourette syndrome (TS) is a childhood-onset and relapsing disorder characterized by involuntary simple or complex tics and high co-morbidity with behavioral anomalies. Its pathophysiologic mechanisms remain unclarified. We investigated immunologic alternations and serum heavy metal levels in patients with TS to elucidate the unclarified mechanisms. Based on the Yale Global Tic Severity Scale, fifteen TS subjects (four females) aged 8–34 (mean: 15.4 ± 6.7) in exacerbation with mean severity score 40.3 ± 14.6 were enrolled in this study. The immunoglobulin levels were normal except for higher immunoglobulin E levels (in 10 patients) with atopy. In exacerbation, there were reverse CD4/CD8 (in two), higher percentages of natural killer cells (in five) and memory T cells (in eight), diminished lymphocyte activation CD69 marker (in three) and impaired NK cytotoxicity (in six) that showed a trend of lower inhibitory CD94 (NKG2A), activating NKp46, and perforin expression compared to those of patients with stable TS and healthy controls, but similar granzyme expression. Serum ASLO, mycoplasma antibody and the levels of heavy metals were not significantly different. All aforementioned immune alterations returned to the normal ranges except for the consistently higher memory T cells. Our study demonstrated that, in some patients with TS, consistently higher memory T cells and lower cytotoxicity in exacerbation status reflect immune alterations and underscore the potential for immunomodulation or immunosuppressive treatment.

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Tourette syndrome (TS) is a childhood-onset, chronic, relapsing disorder characterized by involuntary simple or complex motor and vocal tics and high co-morbidity with behavioral anomalies such as obsessive-compulsive disorder (OCD) and attention-deficit-hyperactivity disorder (ADHD) (1). Its patho-physiologic mechanisms may combine genetic and environmental factors but remains unclarified (1). Two common infectious pathogens of group A β -hemolytic streptococci (GABHS) and mycoplasma pneumonia can be thought as the environmental factors and trigger immune-mediated TS processes. GABHS infection can produce anti-neuronal antibodies against putamen and

enhance B lymphocyte antigen D8/17 expression for effector cells to attack (2). Pediatric autoimmune neuro-psychiatric disorders associated with streptococcal infection (PANDAS) proposed by Swedo et al., represents a distinct clinical entity that may have manifestations of TS, OCD, and ADHD (3). The PANDAS hypothesis currently agrees on the serum Anti-Streptolysin O (ASLO) level in Italian patients with TS (4). Regarding to Mycoplasma pneumonia, the common cause of respiratory infection, it worsens the clinical symptoms of patients with TS (5). Central nervous system (CNS) complications of mycoplasma infection are often seen without proceeding or associated pulmonary symptoms (6).

Following GABHS and mycoplasma infection, the immune offense shares molecular mimicry between allo-antigens (from streptococcal or mycoplasma infections) and self-antigens in the basal ganglia neurons of the CNS to disturb the cortico-striatal-thalamic-cortical circuitry, resulting in motor symptoms such as tics, dystonia, or Parkinsonism (2, 7, 8).

Aside from infection, patients with metal storage disorders because of genetic mutations, such as Wilson diseases, aceruloplasminemia, and hereditary hemochromatosis, have copper or iron deposits in the basal ganglia and subsequently present as 'tics' or other movement disorders (9).

This study investigated the immune alterations in exacerbated and stable TS, possible infectious etiologies of GABHS and mycoplasma pneumonia, and heavy metal deposition. Genetically, a possible candidate gene of the Silt and Trk-like family member 1 (SLITRK1) (10) was also examined in Taiwanese patients with TS.

Subjects and methods

Disease status and severity

The severity of TS was measured using the Yale Global Tic Severity Scale (YGTSS) while the symptoms were evaluated through a best estimate consensus procedure performed by two experienced child neurologists from January 2005 to December 2006. All of the subjects met the DSM-IV criteria for TS diagnosis. The term 'exacerbation' designated those with score of 19 or higher, or a monthly increase of 9 in the YGTSS. If patients had TS and OCD, the term denoted those with monthly increase of 33 score YGTSS plus Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS) or monthly increase 14 score Δ YGTSS plus Δ CY-BOCS. Otherwise, the status was considered stable (11).

Immunologic assessment, infectious pathogens and serum heavy metal level

After obtaining international review board (IRB) approval and informed consents, 20 ml peripheral blood from the patients with TS and age-matched controls was taken. To induce lymphocyte proliferation, peripheral blood mononuclear cells (PBMCs) (10^5 /well) were incubated with the indicated concentrations of Phytohaemagglutinin (PHA), concanavalin-A (ConA), and pokeweed mitogen (PWM) for three days, or the Candida antigen, BCG vaccine, and tetanus toxoid for 7 days, as previously described (12). Serum immuno-globulin, anti-nuclear anti-

body (ANA), ASLO, mycoplasma antibody, Cu, ceruloplasmin, iron, ferritin and Hg levels were evaluated. Lymphocyte subsets (all antibodies purchased from Pharmingen, San Jose, CA, USA), including CD3+, CD4+, CD8+, CD19+, CD45, CD45RO+, CD45RA+, CD27+, CD16+CD56+ (natural killer), and CD69, were assessed by flow-cytometry.

Cytotoxicity

Effector and target cells, K562, were added in 10×10 mm to yield E:T ratios of 25:1, 12.5:1 and 6.25:1 as indicated. Control wells with only target or effectors cells were assayed to determine spontaneous cell death. Cells were mixed by gently tapping, centrifuged at 200 *g* for 1 min and incubated at 37°C in 5% CO₂ for the indicated period of time. After the incubation, 10 μ l of mouse anti-human CD45 monoclonal antibody directly conjugated with fluorescein isothiocyanate (FITC) were added to each tube. The tubes were mixed gently and incubated for 20 min on ice. Twenty microliters of propidium iodine (Sigma, St. Louis, MA) at 1 μ g/ml were added to each tube before acquisition, as previously described (12). Patients with TS in exacerbated and stable status, as well as healthy controls, underwent cytotoxicity functional assessment.

NKp46, CD94, perforin and granzyme expressions in NK cells

The surface expression of NKp46, CD94 (NKG2A), intra-cellular perforin, and granzyme in NK cells were evaluated from fresh PBMCs. TCR $\alpha\beta$ - (PerCP) and CD56+ (PE) gated the NK cells. Briefly, anti-NKp46 and CD94 conjugated with FITC-labeled (Pharmingen) were incubated for 30 min at room temperature. For intra-cellular staining, cytofix/cytoperm (Pharmingen) was used for permeabilization after TCR $\alpha\beta$ - (PcrP) and CD56+ (PE) staining. Anti-perforin and granzyme (Pharmingen) conjugated with FITC-labeled were incubated for 30 min at room temperature. A minimum of 10,000 events were analyzed by flow cytometry.

Candidate gene SLITRK1 sequencing

Candidate gene SLITRK1 was sequenced (10, 13). Each pair of two oligonucleotide primers was designed and selected to cover the entire coding region. The primer sequences were based on human genome sequences that were available upon request.

Results

Patient characteristics

During the study period, fifteen (four females) enrolled TS patients with score over 19 belonged to the exacerbation status and three with lower scores were the stable controls (Table 1). Of the 15 patients with exacerbated TS, 11 (73%) had atopy, which presented as allergic conjunctivitis, rhinitis, eczema, or/and recurrent wheezing. Eight had co-morbidity with OCD or/and ADHD. Of the three stable TS controls, two had atopy, one had OCD, and another one had ADHD. Treatment included dopaminergics, serotonergics, noradrenergics, CNS stimulants, and neuropsychiatric drugs but was individualized (Table 1). Three patients (T9, T11, and T12) did not take medications and handled their TS symptoms on their own. All patients without detectable ASLO and mycoplasma antibody did not receive any anti-streptococcus or anti-mycoplasma antibiotics.

Immunologic assessment, infectious pathogens and serum heavy metal levels

There were higher levels of immunoglobulin E (IgE) in 10 patients and higher immunoglobulin G (IgG) in three patients (Table 2). Lymphocytic proliferation function, ANA, ASLO, mycoplasma antibody, serum Cu, ceruloplasmin, iron, ferritin, and Hg were all within normal range (data not shown). In exacerbation, there were more memory T cells (in eight patients) and more NK cells (in five), decreased 'activated' CD69+ lymphocytes (in two) and reverse C4/CD8 (in two) of which returned to the normal ranges in stable status except for the consistently higher memory T cells.

Cytotoxicity and NKp46, CD94, perforin and granzyme expression in NK cells

After incubating with the K562-tumor cell line, cytotoxicity was estimated by PI staining. Compared with three patients with stable TS and 10 healthy controls, there was decreased cytotoxicity in six patients (T1, T6, T7, T8, T10, and T14) with exacerbated TS, but not closely related to the severity scores of YGTSS. The surface expressions of activating NKp46 and inhibitory CD94 on the patients' NK cells decreased in five (T6, T7, T8, T10, and T11) and 6 (T4, T5, T6, T9, T10, and T11) patients, respectively. Intracellular perforin expression also diminished in two patients (T4 and T6), but granzyme expression was within normal in all (Table 3). Impaired

cytotoxicity, NKp46, CD94, and perforin expressions in some patients with exacerbated TS were restored when they became stable (data not shown).

SLITRK1 sequencing

There was no SLITRK1 mutation identified in the study patients. More comprehensive genetic analysis of SLITRK1 including promoter and the 5' and 3'-untranslated regions in 160 Taiwanese patients with TS was consistently negative finding (14).

Discussion

Dysregulation of the cortico-striatal-thalamic-cortical circuitry, particularly in the basal ganglia and including the caudate nucleus and the inferior prefrontal cortex, is rationally responsible for the development of TS. However, such hypothesis is not always corroborated by post-mortem neuro-pathology and geographic regions (15). The treatment strategy popularly concentrates on correcting the deviated circuitry, mainly targeting only dopaminergic hyper-innervation (dopamine blockers) or in combination with serotonergics, noradrenergics, CNS stimulants, or neuropsychiatric medication (16, 17). Some patients with refractory or malignant TS responded not so well to these combination and antibiotics, even though the evidence of antibiotics to eradicate pathogens and attenuate autoimmunity warrants further investigation (16–18). In practice, plasmapheresis and intravenous immune globulin (IVIG) used to treat patients with systemic lupus erythematosus who suffered from refractory or/and overwhelming autoimmune disasters involving the blood, kidneys, or CNS, have also benefited children with severe TS and OCD (19). This is particularly true for self-injurious behavior (SIB) in TS cases with motor tics that cause cervical myelopathy (20), or the so-called malignant TS (21). As such, this immunologic analysis aimed to explore the unclear pathogenesis of patients with TS.

Among exacerbated patients with TS, seventy three percent (11 of 15) had atopy, of which allergic rhinitis was predominant in all with higher serum IgE. A vicious cycle seemed to exist between an allergic attack and the waxing TS episode (22) because the uneasy state of allergic flare-up proceeded or paralleled the TS episode or exacerbation. Two of three patients with elevated IgG had reverse CD4/CD8, possibly reflecting recent post-infectious immune

Table 1. Clinical manifestations, co-morbidity, and treatment of patients with Tourette syndrome (TS)

Patient Sex	Age (years)		Yale scale score*				ADHD	Allergic disease	Treatment			
	Onset	Now (tested)	Motor tics	Vocal tics	Total injury	OCD			Schizophrenia	Dopamine blocker	CNS stimulants	Serotonergics
Exacerbation												
1 F	8	21	18	13	10	Yes		AC, AR, Ecz	Ami	Ris		Par
2 M	6	7	13	18	10		Yes					
3 M	14	15	19	15	40		Yes	AC, AR, wheeze, Ecz		Sin		
4 F	9	12	8	14	10			AC, AR, wheeze, Ecz		Sin, Hal		
5 M†	5	9	11	8	20		Yes	AC, AR, wheeze, Ecz			Met	
6 M	10	18	8	6	10		Yes	AC, AR				
7 M	5	34	21	18	10			AR	Ami	Sin, Hal		Par
8 F	10	15	17	9	20			AC, AR, Ecz	Top, Sul			
9 M	4	9	16	11	30		Yes	AR, wheeze				
10 M	9	15	7	15	30		Yes	AC, AR, wheeze, Ecz	Top	Ris, Bup		Ven
11 F	7	14	9	3	10		Yes					
12 M	10	19	11	10	10							
13 M	7	18	0	16	20							
14 M	7	9	11	0	10			AR, Ecz	Sul	Ris, Per		
15 M	12	19	4	14	30			AR, wheeze	Top	Ris		
Stable												
16 M	9	21	4	3	0		Yes	AR, wheeze				
17 F	6	18	6	6	0			AC, AR				
18 M	11	24	0	13	0		Yes					

ADHD, attention-deficit-hyperactivity disorder; OCD, obsessive-compulsive disorder; Ris, risperidone; AC, allergic conjunctivitis; AR, allergic rhinitis; Ecz, eczema; Ami, amisulpride; Par, paroxetine; Clo, clonidine; Tri, trihexyphenidyl; Sin, sinemet; Bup, bupropion; Hal, haloperidol; Met, methyphenidate; Top, topiramate; Sul, sulpride; Ven, venlafaxine; Per, perolide; CNS, central nervous system.

*Yale Global Tic Severity Scale.

†His parents has TS.

Table 2. Immunoglobulin levels and lymphocyte subsets in patients with Tourette syndrome (TS)

Patient sex	Yale scale score	Igs (mg/dl except IU/ml in IgE)				Absolute lymphocyte count (mm ³)	Lymphocyte subsets percentages (%)							Activated lymphocyte†
		IgM	IgA	IgE*	IgG		CD4	CD8	CD19	CD16 CD56	Memory cell†			
											T-	B-		
Exacerbation														
1 F	41	89	241	2	1310	1960	42.3	31.5	14.2	10.1	38.2	16.1	86.0	
2 M	31	180	279	26	1630 (1167)	2960	28.9	28.5	13.5	17.9	30.7	19.5	72.7	
3 M	74	48	288	90	<u>1890</u> (1257)	3020	24.8 (31.2)	30.2 (29.4)	14.8	21.6	51.4 (54.3)	14.2	65.7	
4 F	32	81	159	525	1210	2000	32.2	31.2	17.7	15.9	43.7 (47.1)	16.0	75.8	
5 M	39	85	277	<u>182</u>	1290	2870	27.3	27.1	10.5	24.3 (18.7)	33.5	27.4	89.4	
6 M	24	64	251	<u>741</u>	1100	2040	32.1	19.0	12.6	32.6 (21.5)	34.0	15.1	57.7 (82.7)	
7 M	49	117	208	<u>886</u>	1030	2090	38.8	29.3	13.2	16.4	45.7 (42.8)	27.8	74.2	
8 F	46	117	139	14	994	2870	37.8	28.4	15.6	13.1	30.4	21.4	47.1 (75.8)	
9 M	58	166	177	870	<u>1684</u> (1343)	2480	25.7 (32.4)	39.2 (31.8)	8.9	24.4 (19.7)	40.0 (45.2)	27.8	84.5	
10 M	52	189	238	<u>1085</u>	1300	3080	47.1	25.4	12.7	16.2	54.2 (57.8)	30.8	77.9	
11 F	22	189	85	179	1200	2080	47.7	20.5	9.9	10.3	31.2	20.0	92.6	
12 M	31	67	210	188	1240	2890	36.7	32.6	10.9	20.0	41.2 (44.7)	23.4	85.6	
13 M	36	86	139	13	967	1430	27.4	21.8	13.6	30.6 (22.4)	57.1 (53.4)	31.2	48.0 (81.4)	
14 M	21	127	168	<u>1767</u>	867	1730	27.6	20.3	13.3	25.3 (19.4)	47.7 (41.7)	24.8	75.5	
15 M	48	86	199	<u>61</u>	1230	2290	35.4	33.1	18.2	14.8	35.2	21.7	85.4	
Stable														
16 M	7	98	86	<u>475</u>	987	2150	32.5	22.8	12.4	8.4	22.5	19.2	91.5	
17 F	12	107	121	<u>857</u>	1172	1980	42.6	31.7	10.7	13.7	28.7	25.4	87.4	
18 M	13	113	97	<u>57</u>	1440	2420	51.4	32.8	8.8	7.5	16.8	18.7	75.7	
Healthy controls		56–352	70–312	25–1376	639–1349	1250–3100	27–56	12–35	6–20	3–23	2–38	3–32	67–94	
n = 12														

TS patients who recovered from the exacerbation status to the stable status have restored lymphocyte subsets in parentheses (except the memory T-cell subset). Thick and bold numbers and underlying lines mean over or beyond normal ranges.

Ig, immunoglobulin.

*The non-atopic references of IgE by age are <90 (6–9 years), <200 (10–15 years) and <100 IU/ml (adult) in [Ref. (37)].

†T-memory percentage = CD3+CD45RO+/CD3+CD45RO– and CD3+CD45RO– or CD3+CD45RA–/CD3+CD45RA– and CD3+CD45RA–/CD3+CD45RA–; B-memory percentage = CD19+CD27+/CD19+CD27– and CD19+CD27–; Activated lymphocyte percentage = CD3+CD69+/all lymphocytes.

Table 3. Cytotoxicity to the K562 cell line and the expression of NKp46, CD94, granzyme, and perforin expression in patients with Tourette syndrome

Patient	Yale scale score	Cytotoxicity			NK cells (gated by CD16+CD56+ or CD56+TCRαβ−)			
		Effector to target cell ratio			Percentage (mean fluorescent intensity)			
		25/1	12.5/1	6.25/1	NKp46	CD94	Granzyme	Perforin
Exacerbation								
T1	41	12.2%	10.3%	6.3%	73.1% (25.3 ± 17.0)	88.0% (25.6 ± 43.4)	ND	81.3% (32.4 ± 22.3)
T2	31	57.8%	38.2%	17.6%	81.1% (28.3 ± 18.6)	84.5% (36.3 ± 21.6)	ND	56.0% (20.8 ± 17.9)
T3	74	60.0%	54.9%	46.7%	67.1% (24.2 ± 17.0)	83.1% (29.3 ± 21.4)	ND	63.1% (23.7 ± 15.5)
T4	32	ND	23.7%	36.3%	39.6% (16.9 ± 16.2)	61.8% (22.7 ± 24.4)	68.5% (29.4 ± 13.2)	13.5% (12.4 ± 11.5)
T5	39	31.7%	48.0%	ND	64.7% (24.1 ± 16.5)	57.8% (20.3 ± 26.2)	56.7% (27.8 ± 13.2)	40.0% (19.2 ± 12.0)
T6	24	22.8%	18.6%	9.1%	22.3% (13.2 ± 18.9)	59.1% (22.5 ± 30.4)	ND	23.2% (14.7 ± 11.4)
T7	46	15.0%	9.8%	4.8%	23.2% (13.3 ± 17.2)	86.4% (33.4 ± 13.8)	47.1% (24.3 ± 14.5)	67.9% (25.5 ± 13.3)
T8	49	9.6%	7.7%	2.7%	25.2% (14.0 ± 21.8)	68.4% (28.5 ± 20.1)	62.8% (29.4 ± 17.4)	95.9% (49.6 ± 15.7)
T9	58	55.6%	49.6%	46.9%	30.9% (14.8 ± 18.5)	57.4% (17.5 ± 19.8)	72.5% (20.8 ± 16.1)	88.1% (44.1 ± 18.3)
T10	52	13.2%	4.9%	ND	12.5% (12.3 ± 12.3)	26.9% (11.9 ± 14.3)	45.8% (16.1 ± 19.0)	51.7% (22.6 ± 13.2)
T11	22	41.3%	45.0%	38.0%	26.9% (15.9 ± 14.3)	60.3% (22.5 ± 19.9)	73.5% (23.4 ± 12.2)	96.1% (83.9 ± 39.1)
T12	31	58.9%	52.9%	30.3%	ND	ND	ND	ND
T13	36	73.8%	62.4%	41.8%	ND	ND	ND	ND
T14	21	20.9%	12.5%	ND	52.9% (19.4 ± 17.3)	71.5% (35.0 ± 27.4)	59.5% (23.1 ± 18.5)	97.5% (84.2 ± 57.2)
T15	48	28.0%	26.2%	34.7%	68.9% (20.4 ± 14.6)	71.1% (41.9 ± 33.9)	88.4% (38.0 ± 25.1)	97.5% (79.4 ± 59.6)
Stable								
T16	7	19.9%	13.9%	18.2%	45.6% (15.8 ± 13.3)	86.9% (32.7 ± 22.5)	ND	48.8% (18.4 ± 17.4)
T17	12	33.3%	26.2%	13.3%	60.0% (21.0 ± 18.6)	66.0% (22.0 ± 23.1)	81.2% (32.4 ± 18.2)	39.9% (17.3 ± 14.1)
T18	13	49.9%	57.4%	40.5%	50.6% (16.0 ± 14.3)	80.1% (47.8 ± 19.5)	62.8% (18.4 ± 15.6)	68.6% (26.1 ± 19.3)
Healthy controls								
n = 10		26.1–78.9%	20.4–57.9%	15.5–50.3%	30.8–83.5% (14.3–36.8)	64.4–91.5% (25.4–42.0)	34.4–93.1% (13.8–54.2)	39.4–92.7% (18.7–84.5)

Thick and bold numbers and underlying lines mean beyond normal range in exacerbation but restored normal in stable status (data not shown).
 ND, not performed.

response, although the negative evidence of the common pathogens of GABHS and mycoplasma. The higher memory T cell subsets (54.5%; eight of 15 patients) in both the exacerbation and stable status revealed that the consistent activation signaling primed naive T cells through the interaction of antigen-presenting cells and naive precursor frequency, subsequently to expand the memory T cells pool (23). However, there was relatively decreased 'activated' (CD69+) lymphocytes in two patients, revealing the existence of not only activate but also inhibitory signaling in the heterogeneous and complex TS mechanisms.

Six exacerbated patients had decreased cytotoxicity to K562 cell lines with decreased 'recognizing' surface activating NKp46 receptor (in T6, T7, T8, and T10), inhibitory CD94 receptor (in T6 and T10) or/and intra-cellular perforin expression (T6) in NK (TCR $\alpha\beta$ -CD56+) cells. Similar patterns appeared on the natural killer T (NKT) cells (TCR $\alpha\beta$ +CD56+) and were restored in the stable status (data not shown). Diminished inhibitory CD94 expression (in T4, T5, T9 and T11) was potential to counterbalance the effect of either lower NKp46 (26.9% in T11) or lower perforin (13.5% in T4), but neither both (in T6) nor extreme low NKp46 expression (12.5% in T10), subsequently to maintain the normalized cytotoxicity. In contrast, the decreased cytotoxicity in T1 and T14 patients could have normalized the CD94, NKp46, and perforin expression. Such paradoxical observations implied unknown compensatory and complicated pathways involved NK cytotoxicity in patients with TS. However, impaired NK cytotoxicity is one of the diagnostic criteria for hemophagocytic lymphohistiocytosis (HLH) from cytokine storm and presents as an aberrant and dysregulated higher TNF- α and IL-12 resembling patients with TS (24). Immuno-modulation or/and immuno-suppressive drugs, including cyclosporin, steroids, episode, and IVIG recommended in HLH treatment protocol, might have the potential effect to restore impaired cytotoxicity in exacerbation (25) and overcome memory T-cell-mediated responses (26).

We further investigated the effect of the serum levels of heavy metals and diet contents on TS. As literature review, one boy using Chinese herbal spray with mercury intoxication (27), two first-degree teenage cousins taking caffeine-rich food, like chocolate (28), have been reported to develop tics that completely disappeared after withdrawal of these substances. Of nutrition factors, the serum ferritin level positively affects on the putamen volume. Patients with relatively

lower ferritin level, subsequently relatively small volume of putamen, are prone to tic development or exacerbation (29). The levels of associated heavy metals, including Hg, copper, ceruloplasmin, iron and ferritin, are all within normal limits. To exclude the unintentional diet contents and habits, caffeine restriction is not able to suppress TS movement in the study patients. These argue against the significant relationship.

Genetically, two Caucasian male patients with TS have been noted with heterozygous mutation of SLITRK1 encoding a single-pass trans-membrane protein with two leucine-rich repeat motifs in its extra-cellular domain. This mutation involves disturbed dendritic growth and neuro-anatomic circuits (30). Sequencing the candidate gene SLITRK1 in 90 Austrians (15), 18 in this study and 160 more Taiwanese (14) TS patients remains indeterminate and argues the pathogenic role of SLITRK1 in other people although selection bias may exist. Poly- or oligogenetic inheritance on chromosomes 4, 5, 8, 11, and 17 instead of monogenetic SLITRK1 mutation encompass such a phenotype that typically decreases in severity with age, and with symptoms that overlap with common co-morbidities of OCD and ADHD (31–36).

In conclusion, immune alterations do develop in the subgroup of Taiwanese patients with TS. Impaired cytotoxicity in exacerbation and consistently higher memory T-cell subsets may play potential roles in elucidating the unclear pathogenesis. This study also highlights the possibility of using immuno-modulation or immuno-suppression for the subgroup of refractory or malignant TS.

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